EXHIBIT B15
Part 2

Copy Produced by Plaintiffs Prior to Deposition

Personal Information

Black n' Red

Use this page as a quick reference to the book's contents during use and for archiving purposes

9/26/2017

- Cell lines

SKOV-3	ATCC
A2780	Sigma Aldrich, St. Louis, MO
TOV112	A kind gift from Genshong Wu at Wayne State Un

EL-1 / macrophages

Normal Ovarian epithelial Cell Biologic, Chicago, IL

- Fetal bovine serum (FBS, Innovative Research, Novi, MI)
  Penicilin/streptomycin (Fisher Scientific)

- Talc (Fisher # T4-500 Lot # 166820)

28

Seeded Cells for PCR

9/26/2017

— Thawing Cells

	Media
EL-1 (Macrophages)	IMDM (10% FBS, 1% PS, 1ml H-T, 2μL
Normal Ovarian Epithelial	Complete Human Epithelial Cell medium k (Cell Biologics)
SKOV-3	McCoy's 5A (10% FBS, 1% PS)
TOV112	Medium 199: MCDB 105 (1:1) + 10% FBS +
AL780	RPMI-1640 (10% FBS + 1% PS)

75 cm² flask + 15 mL medium

9/29/2017

— Subculture cells

※ Check ~~to~~ under microscop cells are 70~80% full.

① Sork out old medium
② Wash with 10mL PBS
③ gently remove PBS
④ pipet trypsin-EDTA 2mL onto the washed Cells mondarger
※Normd Ovarian Epithelial use trypsn from ScienCell
⑤ 37°C incubator 1~5 minutes (SKOV-3 longer)
⑥ Check under microscope
⑦ Add fresh medium 8mL to inactive trypsin, Then mix
⑧ Take 2mL to a new 100mm dish
⑨ Add 8mL Fresh medium to 100mm dish
⑩ Incubate the cells

※ One time ~~treat~~ ~~onecell~~ One Cell line.

10/3/2017

- Subculture Cells

2mL Cells + 8mL medium 100 mm dish

Cells doubled in one day

10/6/2017

- Subculture Cells

- Seeded Cells for talc treat

1×10^6 cells/dish 60mm dish + 5mL medium

10/7/2017

- Treat cells with talc

Prepare talc

100mg talc + 10ml DMSO → mix 10 mg/mL = 10^4 µg/mL

- sterilization under UV light to avoid endotoxin and microbial Contamination

(x_1) (10^4 µg/mL) = (5mL) (20 µg/mL) → $x_1 = 10$ µL

(x_2) (10^4 µg/mL) = (5mL) (100 µg/mL) → $x_2 = 50$ µL

(x_3) (10^4 µg/mL) = (5mL) (1000 µg/mL) → $x_3 = 500$ µL

10/10/2017
After 72 hours treatment

— Collect cells

Cell Collection Protocal

Put on gloves and spray with 70% ethanol
Remove cell culture dish from incubator
Observe cells under microscope.
Move the dishes to your work bench, does not need to be done in the hood.
Collect media and place in labeled 15ml tube for freezing,
Add 10 ml PBS
Using a cell scraper, scrape the bottom of the dish and rotate it to ensure scraping of entire bottom
Using a 10ml pipet, remove the PBS and cell mixture and place into the 15ml conical centrifugation tube that corresponds to the dish, 1ml for RNA, 2ml for DNA,8 ml for protein assay.
Close and centrifuge all tubes, 5 minutes at 1800rpm (slower speed keeps cells from breaking). Place another paper towel by sink, dump PBS from all tubes into sink and place tubes upside down to drain them. Cells will be collected at the bottom. Place all tubes in Styrofoam holder and place in -80°C freezer.

— RNA Extraction
RNeasy Mini Kit (Qiagen Cat # 74106) (go to pg 42. 43)

— Detect Concentration of RNA by Nanodrop
(Thermo Fisher Scientific)
(go pg 43)

— cDNA Synthesis via Reverse Transcription — VILO kit
Life Technolog
(go pg 43)

RNA Extraction

RNeasy Mini Kit (Qiagen cat # 74106)

Important Notes before starting: WORK IN THE HOOD

- β-Mercaptoethanol (β-ME) can be added to Buffer RLT (lysis buffer) before use. β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10 µl β-ME per 1 ml Buffer RLT. Buffer RLT is stable for **one month** after addition of β-ME.
- Buffer RPE is supplied as a concentrate. Before using for the first time, add ethanol as indicated on the bottle. Be sure to **mark the lid with a X** to show that the working solution has been prepared.

Buffer RW1 and Buffer RLT are hazardous.

- **Buffer RLT+ β-ME should be disposed of in the jar in the hood.**
- **Buffer RWI should be disposed of in the jar in the hood.**

Preparation of the Buffer RLT

- In a labeled 15ml centrifugation tube, add 10µl β-ME for every 1 ml Buffer RLT.

Preparation of your samples

1. Add 350 µl of the Buffer RLT + β-ME solution to each of your sample tubes.
   a. if you have a lot of cells, you will need to add 600 µl of Buffer RLT + β-ME solution to each tube ***also add equal volume of ethanol)
2. Add 350 µl of 70% ethanol to each tube and pipet to mix
3. Transfer the entire sample to its corresponding mini spin column
   a. Close columns and place them into the small centrifuge.
   b. Centrifuge the tubes for 15 seconds at 13,000 rpm
4. Dump the flow through into hazardous waste jar **in the hood**.
5. Add 700µl of the Buffer RW1 to the RNeasy column
   a. Centrifuge 15 seconds at 13,000 rpm
6. Dump the flow through into hazardous waste jar **in the hood**
7. Add 500µl of Buffer RPE onto each RNeasy column
   a. Centrifuge 15 seconds at 13,000 rpm
8. Dump the flow through into waste jar
9. Add 500µl Buffer RPE to each column again
   a. Centrifuge 2 minutes at 13,000 rpm to dry the silica gel membrane
10. Dump the flow through in waste jar, centrifuge for one minute more
11. Remove columns from collection tubes and place in corresponding 1.5ml centrifuge tube
12. Add 50µl of RNase-free water to each column, onto the center of the silica-gel membrane without touching the sides of the column (water dissolves RNA).
    a. Allow to stand for 1 minute
    b. Centrifuge columns for 1 minute at 13,000 rpm, LID MUST BE ON CENTRIFUGE
13. Collect flow through from the collection tube and place back into the column on the center of the membrane, allow to stand for 1 minute
    a. Centrifuge columns again for 1 minute at 13,000 rpm, LID MUST BE ON CENTRIFUGE
14. Remove and dispose of columns
15. Place your microcentrifuge tubes containing RNA on ice
    a. Detect concentration of RNA
    b. Good quality RNA has a A260/A280 of 2.0

NEED TO MEASURE RNA EACH TIME YOU GO TO MAKE cDNA

cDNA Synthesis via Reverse Transcription

You will need:
Ice
Thaw, on ice:
RNA
VILO MasterMix
RNase-free water

You must detect the concentration of your RNA. After doing this, you can calculate the volume needed to get for a 1 µg reaction.
i.e. – If your RNA concentration is 0.9 ug/ul then:
(x ul)(0.9 ug/ul) = 1 ug solve for x

For a single reaction, combine the following components in a sterile PCR tube on ice.

	1 µg RNA
Component	**Volume/reaction**
VILO MasterMix	4 µl
Template RNA	Variable up to 1 µg
RNase-free Water	Variable
Total Volume:	20 µl

The total amount in each tube should equal **20 ul**, hence the variable volume of water.

- Add 4 ul VILO MasterMix to each tube, volume of RNA calculated, volume of water calculated, and gently mix.
- Place the tubes in a rack and the rack into a 25°C water bath for 10 minutes.
- Place the rack into a 42°C water bath for 60 minutes.
- Then, place racked tubes into 85°C water bath for 5 minutes to terminate the reaction.
- Place samples on ice for a few minutes.
- Centrifuge cDNA.
- Place into -80°C freezer for storage or continue on.

Do 0.2ug Reaction

Sample	Concentration ug/ul RNA	ul RNA for 0.2 ug in 1.5 ug rxn	ul Water
SKOV unt 72 hr	0.0521	3.8	20.2
SKOV talc 20ug/ml 72 hr	0.0431	4.6	19.4
A2780 unt 72 hr	0.0976	2.0	22.0
A2780 talc 20 ug/ml 72 hr	0.1067	1.9	22.1
EL1 72 hr	0.0067	24.0	0.0
EL1 talc 20ug/ml 72 hr	0.0146	11.0	13.0
SKOV talc 100ug/ml 72 hr	0.086	2.3	21.7
SKOV talc 1000ug/ml 72 hr	0.0592	3.4	20.6
A2780 talc100ug/ml 72 hr	0.0289	6.9	17.1
A2780 talc 1000ug/ml72 hr	0.0335	6.0	18.0
EL1 talc 100ug/ml 72 hr	0.0104	15.5	8.5
EL1 talc1000ug/ml 72 hr	0.0128	12.6	11.4
Normal OV Epi 72 hr	0.0433	4.6	19.4
Normal OV Epi talc 20ug/ml72 hr	0.0385	5.2	18.8
Normal Ov Epi talc 100ug/ml72 hr	0.0357	5.6	18.4
Normal Ov Epi talc 1000ug/ml72 hr	0.0667	3.0	21.0

0.2ug RNA was obtained from each sample following dilution as described by this table.

cDNA (30ul) prepared

43

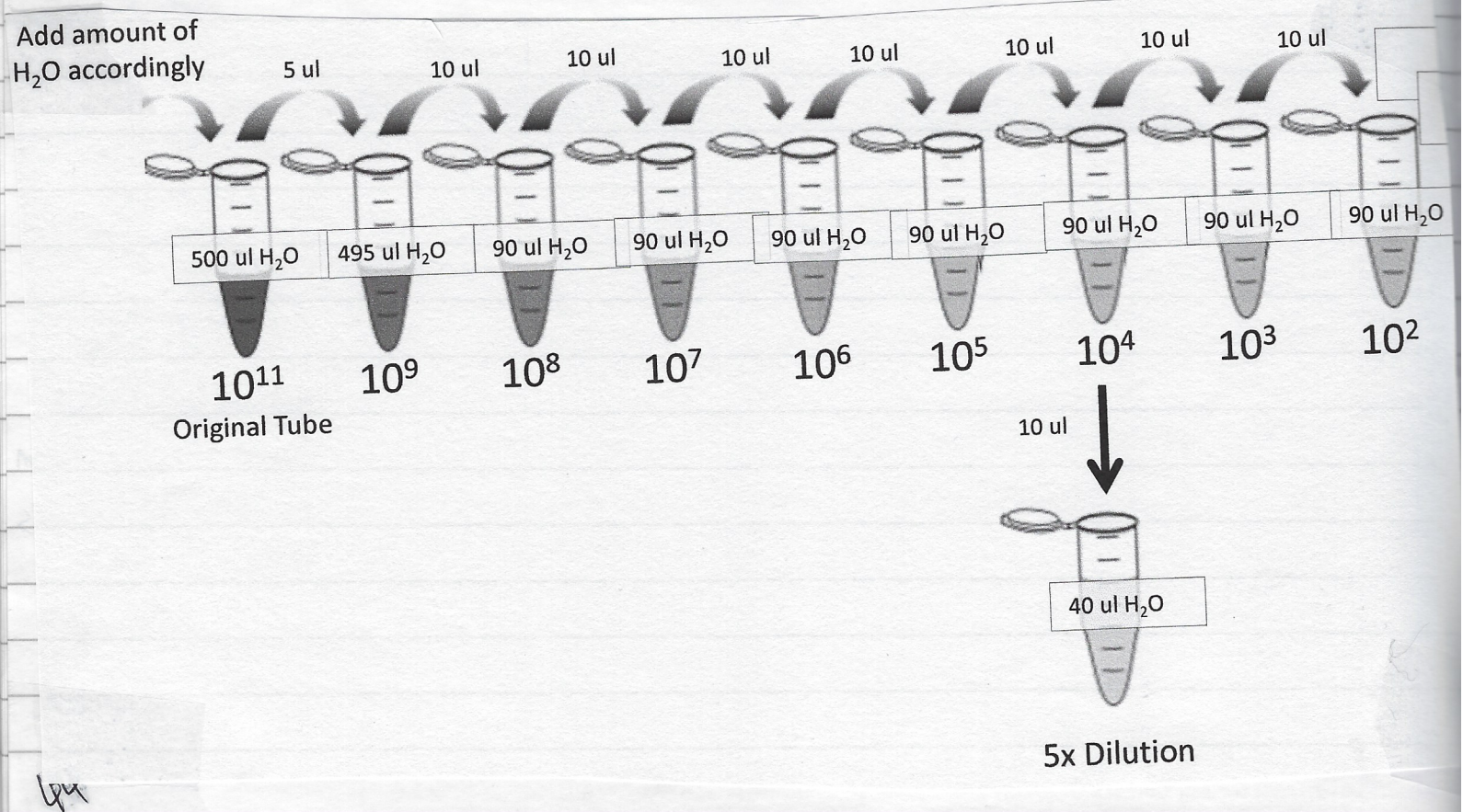
10/11/2017 Real-time PCR for β-actin

β-actin – Standard

– Standards come desiccated

- Reconsitute the standard using TE buffer
- The volume of TE buffer is on the product sheet
  ① You will add TE buffer such that the concentration will be 100μM
  - Mix well
  ② In a new 1.5mL microtube, add 5μl of standard to each tube
  - Calculate the number of tubes needed by dividing the volume of TE buffer you added by 5
  ③ put tubes into the concentrator machine for 2aminutes – Lids open
  ④ Close tubes, label the lid with the type of standard and date
  - The box should state that user add 500μl of PCR water to get a standard that is 10^{11}

– Serial Dilution of Standard (place samples on ice after mixing)



Run β-actin with samples

— Do 25µl reaction

Water	9.5µl
Primer Forward	1µl
Primer Reverse	1µl
SYBR Green	12.5µl
Sample (cDNA)	1µl

5µM 20x dilution

→ Radiant Green Lo-Rox qPCR Kit #QS1050

— Calculating Master mix for samples

20 samples X 3 (triplicated) + 1 blank = 61
61 X 1.17 extra = 71.37

— Master mix calculation

Water = 9.5 X 71.37 = 678.015µL = 678µl
primer = 1 X 71.37 = 71.4µL
SYBR Green = 12.5 X 71.37 = 892.125 = 892.1

— Mix, then take out 80.6µl of this mix → 1.5mL tube/~~per~~ sample
24 X 3 X 1.12 extra = 80.6µl

— Add 3.4µl sample to 1.5mL tube containing master mix
1 X 3 X 1.12 = 1.4µL

— Mix well add 25µl → PCR tube
3 total per sample